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Genetic Variability in the M-Line Stock of *Biomphalaria glabrata* (Mollusca: Planorbidae)

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ABSTRACT: Genetic variation among samples of the M-line stock of *Biomphalaria glabrata* is described. Snail samples from 5 separate research laboratory facilities display differences in allozyme frequencies among 9 of 24 enzymes examined. Genetic variability of M-line snails is discussed relative to the origin and maintenance of the stock and its use as a model in studies of snail–schistosome interactions. Inquiries among investigators maintaining and using snails have indicated that multiple albino stocks of *B. glabrata* are or have been used for snail–schistosome research. Exchange of these stocks among investigators and subsequent interbreeding are the most probable explanations for the patterns of genetic divergence observed.

KEY WORDS: *Biomphalaria glabrata, Schistosoma mansoni,* genetic variability, allozymes.

The M-line stock of *Biomphalaria glabrata* is among the snail stocks most often used in studies of snail–schistosome compatibility (Richards and Merritt, 1972; Richards, 1973, 1975, 1984), molluscan immunobiology (e.g., Loker et al., 1987; Noda and Loker, 1989; see references in Bayne, 1983), and where large numbers of infected snails are needed as in vaccine research (Stirewalt et al., 1983). Newton (1955) established the M-line stock to combine albinism and a high level of susceptibility to a Puerto Rican strain of *Schistosoma mansoni.* The derivation of the M-line stock involved a cross between a pigmented Puerto Rican snail susceptible to this stain of *S. mansoni* and an albino Brazilian snail not susceptible to this parasite. Ten generations of inbreeding (selfing) and selection for susceptibility yielded an albino stock that was >90% (although usually not 100%) susceptible to a Puerto Rican *S. mansoni* strain. Additionally, there may have been strong (although inadvertent) selection for early reproduction, because the first individual to reproduce was used to found the subsequent generation. The breeding and selection scheme imposed by Newton (1955, p. 528) led to the prediction that the stock should be "genetically rather homogeneous, although it is to be expected that with random breeding and the occurrence of mutations there will be a gradual loss in this high degree of homozygosity." An assumption inherent in the use of laboratory stocks for studies of snail–schistosome compatibility and snail immunobiology is that they are more genetically homogeneous and will provide a more uniform response to experimental manipulation than would field-collected specimens.

Mulvey and Vrijenhoek (1981) provided evidence that M-line snails were not genetically uniform but were unexpectedly variable. Using electrophoretically detectable genetic variation, they estimated a level of heterozygosity following the initial hybridization between the Puerto Rican and Brazilian stocks (although not the original stocks of Newton) to be approximately 0.32. Ten generations of selfing in which a single selfed snail founded each generation and subsequent selection for susceptibility would be predicted to reduce such heterozygosity to 0.0003. These estimates of initial and subsequent heterozygosity are based on several a posteriori assumptions: 1) that the Puerto Rican and Brazilian stocks used to estimate heterozygosity were representative of the original stocks, 2) that reproduction was exclusively by single self-fertilizing snails, and 3) that the M-line stock sampled is a direct descendant of the original stock and has been isolated from other snail stocks since its origin (i.e., no migration). The M-line sample described by Mulvey and Vrijenhoek was more than 100 times more heterozygous than predicted (H = 0.057). These authors suggested the following possibilities to account for the unexpectedly high levels of heterozygosity in their sample: 1) that there was selection to maintain heterozygosity, 2) that the sample was not representative of all M-line material, or 3) that these snails had been inadvertently outcrossed to other laboratory stocks of *Biomphalaria.*

Studies of snail–schistosome compatibility often have reported variation in snail susceptibil-

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ity. Although such variation may have a number of underlying causes, genetic differences among host individuals is undoubtedly involved. The present study was undertaken to extend the investigation of electrophoretically detectable enzyme polymorphism and heterozygosity in the M-line of *B. glabrata.* If electrophoretic markers can be viewed as an indication of overall genomic variation, these studies may suggest the extent to which genetic variation is important in this stock of snails. A second impetus for this study is the observation that natural populations of *Biomphalaria* generally have levels of electrophoretically detectable polymorphism and heterozygosity comparable to or lower than those reported by Mulvey and Vrijenhoek (1981) for the M-line. This laboratory stock resembles naturally occurring snail populations with respect to genetic population structure more than previously expected.

Here we report on electrophoretically detectable genetic variation in the M-line of *B. glabrata* maintained at 5 laboratories in the U.S.A. The following points will be addressed. 1) Is the variation reported earlier limited to the original sample studied or characteristic of the M-line generally? 2) Do M-line snails maintained at different laboratories collectively represent a homogeneous stock?

Materials and Methods

Samples of approximately 30–40 M-line snails were generously provided by the following: E. S. Loker (University of New Mexico, Albuquerque), W. Granath (University of Montana, Missoula), C. Bayne (Oregon State University, Corvallis), F. Lewis (Biomedical Research Institute, Rockville, Maryland), and T. Yoshino (University of Wisconsin, Madison). These investigators also provided information concerning the history and maintenance of each colony.

Methods for starch gel electrophoresis of *B. glabrata* have been described elsewhere (Mulvey and Vrijenhoek, 1981; Mulvey et al., 1988). For the present study, the lithium–hydroxide buffer of Selander et al. (1971) was used for resolution of esterase allozymes; additionally, the esterase-staining solution contained α-naphthyl acetate and β-naphthyl propionate as substrates. Peptidase enzymes were detected using the following peptide substrates: leucylglycylglycine, leucylalanine, and phenylalanylproline.

Data were analyzed using BIOSYS-1 (Swofford and Selander, 1981) and SAS (SAS Institute, 1985). Genetic variation within and among samples was examined using Wright's (1978) *F*-statistics. Rogers (1972) genetic identity values were clustered using the UPGMA (Sneath and Sokal, 1973) and distance Wagner methods (Farris, 1972).

Results

Twenty-four enzymes were examined in the M-line samples, and 15 were invariant. Allozyme frequencies for the variable loci and overall heterozygosity varied markedly among samples (Table 1). Genotypic frequencies within samples differed significantly from expectations under Hardy-Weinberg equilibrium and random mating in 9 of 37 χ^2-tests. All of these deviations reflected a deficiency of heterozygous genotypes. Contingency χ^2-tests indicated that there was significant heterogeneity in allele frequencies among samples at 8 of the 9 polymorphic loci ($P < 0.00002$). Only allele frequencies for Pep-1 were homogeneous among the 5 samples.

The F_{it} value is a measure of correlation among gametes in individuals relative to the entire population (Table 3). The observed value, $F_{it} = 0.482$, indicates an excess number of homozygotes in the total sample relative to that predicted if the entire sample represented a single randomly mating assemblage. The correlation among gametes within individuals in the total sample (F_{it}) is affected by nonrandom mating (heterozygosity measured by F_{is}) and population size (differences among populations, measured by F_{st}). The F_{is} value indicates mean heterozygosity at the local level; again, positive values indicate an excess of homozygous genotypes relative to expectations under random mating. Among-sample variation can be expressed as a percentage, measured by the F_{st}. Therefore, 17.3% of the total genetic variation is attributable to differences among samples.

Figure 1 provides genetic identity values clustered using UPGMA. The cophenetic correlation was 0.85, indicating a good fit at the original data matrix to the diagram.

Discussion

The M-line samples display unexpectedly high levels of genetic variability. Approximately 30% of the loci examined were polymorphic within the samples. Average individual heterozygosities ranged from 0.051 to 0.098. These levels of genetic variation are comparable to levels found in many naturally occurring populations of *Biomphalaria* (Table 2). Our findings confirm previous reports of high levels of genetic variation in laboratory stocks of *B. glabrata* (Mulvey and Vrijenhoek, 1981; Knight et al., 1991). Working with the 10-R2 stock of *B. glabrata,* an

Table 1. Allozyme frequencies for enzymes in colonies of the M-line stock of *Biomphalaria glabrata*. Allozymes are designated A, B, or C for each locus. Fifteen other enzymes were invariant.*

Locus	Loker	Granath	Lewis	Bayne	Yoshino
Aconitate hydratase-2					
A	0.81	0.18	0.28	0.14	0.64
B	0.19	0.62	0.72	0.86	0.36
6-Phosphogluconate dehydrogenase					
A		0.06	0.29	0.61	0.36
B	1.00	0.94	0.71	0.39	0.64
Phosphoglucomutase-1					
A	0.92	0.72	0.84	0.57	0.54
B	0.08	0.28	0.16	0.43	0.46
Esterase-1					
A	0.99	0.53	0.86	1.00	0.51
B	0.01	0.47	0.14		0.39
C					0.10
Esterase-4					
A	0.55	0.97	0.70	0.32	0.75
B					0.03
C	0.45	0.03	0.30	0.68	0.22
Xanthine dehydrogenase					
A					0.15
B	0.92	0.87	0.82	0.67	0.74
C	0.08	0.13	0.18	0.33	0.11
Peptidase-1					
A	0.08	0.09	0.04		0.10
B	0.69	0.60	0.81	0.69	0.71
C	0.23	0.31	0.15	0.31	0.19
Peptidase-4					
A	0.35			0.24	
B	0.65	1.00	1.00	0.76	1.00
Acid phosphatase					
A				0.23	0.26
B	1.00	1.00	1.00	0.77	0.74
Mean sample size	33.0 ± 4.7	31.0 ± 1.3	30.9 ± 1.1	30.3 ± 3.1	34.1 ± 1.2
% Polymorphic loci	29	29	29	33	33
Mean heterozygosity	0.054	0.051	0.063	0.094	0.098

* Aconitate hydratase-1, adenosine deaminase, aspartate aminotransferase-1, aspartate aminotransferase-2, α-glycerophosphate dehydrogenase, hemoglobin, hydroxybutyrate dehydrogenase, isocitrate dehydrogenase, lactate dehydrogenase, malate dehydrogenase-1, malate dehydrogenase-2, malic enzyme, nucleoside phosphorylase, phosphoglucoisomerase, and phosphoglucomutase-2.

M-line derivative, Knight et al. (1991) observed restriction fragment-length polymorphism variation among snails from various laboratories as well as among progeny of selfed snails. Mulvey and Vrijenhoek (1981) reported a high degree of intrastrain polymorphism even in some presumably inbred stocks.

In agreement with the earlier work of Mulvey and Vrijenhoek (1981), levels of genetic variation observed in laboratory populations of snails are not consistent with predictions based on the intensive regime of inbreeding and selection during the derivation of the M-line stock. Given the hybrid origin of these snails and the estimated initial heterozygosity, it might be possible to maintain these levels of heterozygosity by some kind of balancing selection. However, the occurrence of 3 alleles at the Est-1, Xdh and Pep-1

Table 2. Levels of genetic variability in natural populations of *Biomphalaria* compared with *B. glabrata* M-line. N_s = number of samples, N_l = number of loci, P = proportion of polymorphic, H = mean heterozygosity per sample.

	N_s	N_l	P	H	References
B. glabrata					
Puerto Rico	7	26	0.15	0–0.06	Mulvey and Vrijenhoek, 1982
Dominican Republic	2	21	0.19–0.24	0.022–0.027	Mulvey et al., 1988
St. Lucia	2	21	0.09–0.24	0.003–0.037	Mulvey et al., 1988
B. alexandrina					
Qalubia	7	26	0.16–0.19	0.044–0.092	Graven, 1984
B. straminea					
Hong Kong	4	19	0.26	0.056–0.097	Woodruff et al., 1985
B. pfeifferi					
Kenya	12	10	0.00–0.40		Bandoni et al., 1990
B. glabrata					
M-line	5	24	0.29–0.33	0.051–0.098	This study

loci demands an alternative explanation. A single selfing snail could not have more than 2 alleles. There are 2 possible explanations for the presence of the third allele: mutation and migration.

The spread of a neutral allele in a population would be determined by the effective population size and reproductive rate. Although mutation cannot be entirely excluded, it seems unlikely given the relatively high frequencies observed and its necessarily recent origin. For example, the Yoshino stock has been isolated from the other M-line stocks for at most 20 generations. However, a sequence resembling the LINE-1 element of *Drosophila* has recently been described from *B. glabrata* (Knight et al., 1992). The LINE-1 element is a transposable element associated with higher levels of mutation. Migration is another probable explanation. Even with diligent care, movement of snails among aquaria is a possibility during cleaning, feeding, or handling of the colony. All laboratories from which M-line snails were obtained also maintain other stocks of *B. glabrata*.

Table 3. Wright (1978) *F*-statistics for 5 colonies of M-line *Biomphalaria glabrata*.

Locus	F_{is}	F_{it}	F_{st}
ACON-2	0.261	0.474	0.288
PGD	0.323	0.492	0.249
PGM-1	0.384	0.449	0.106
EST-1	0.510	0.627	0.239
EST-4	0.205	0.372	0.210
XDH	0.309	0.351	0.060
PEP-1	0.425	0.437	0.022
PEP-4	0.269	0.425	0.213
ACP	0.967	0.973	0.167
$\bar{x}$	0.374	0.482	0.173

Among-sample differentiation accounted for approximately 17% of the observed variation. This among-sample differentiation is less than that observed between naturally occurring snail populations but considerably more than would be expected from a homogeneous, inbred laboratory stock (Table 4).

The Lewis colony is an uninterrupted descendant of the original Newton Stock (F. Lewis and C. S. Richards, pers. comm.). Snails have been shipped from this colony to initiate other colonies. The Loker colony was secondarily derived from the Bayne colony in 1983 and the Granath colony from the Yoshino colony in 1984. The Granath and Yoshino colonies were periodically supplemented with snails from J. Bruce (University of Lowell, Lowell, Massachusetts). Laboratories maintaining M-line snails identified additional factors that would be expected to reduce heterozygosity and lead to divergence in the M-line samples. Stocks have been reselected for susceptibility (Bayne, every 30–48 mo) and albinism (Richards, early to mid-1970s). Selection would be expected to reduce the number of genotypes in the population. Additionally, although all laboratories report maintaining their M-line stock in numbers greater than 50 individuals, occasional bottlenecks in population numbers have occurred. For example, Bayne (pers. comm.) reported having only 15 M-line

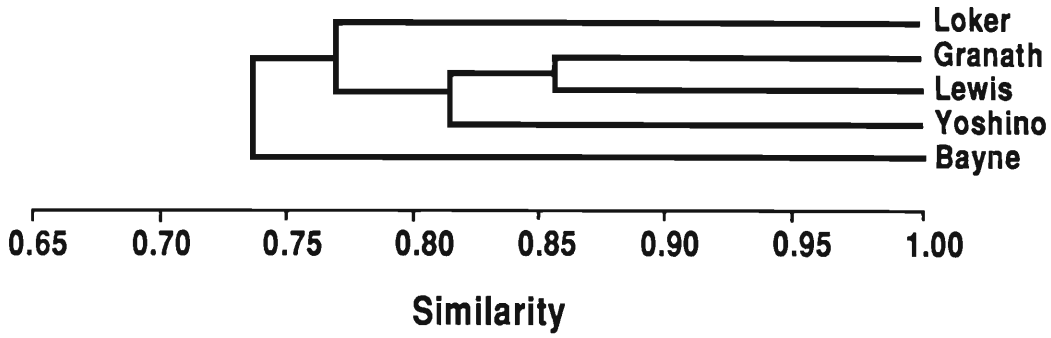

Figure 1. Genetic identity values for 5 colonies of M-line *Biomphalaria glabrata* clustered using unweighed pair group method of averaging. Cophenetic correlation = 0.85.

snails in December 1986. Again, these low numbers might be associated with genetic drift and would be expected to lead to a reduced number of genotypes and differentiation among samples. Finally, *B. glabrata* is a self-compatible hermaphrodite, and although outcrossing is the most frequent mode of reproduction, to whatever extent selfing occurs it would tend to reduce heterozygosity.

Several important points emerge from the data. M-line snails are not genetically homogeneous within populations. Additional genetic differentiation is apparent among populations. It is possible that some contamination of the stock has taken place. The M-line stock is as genetically polymorphic and heterozygous as any natural population of *Biomphalaria* examined to date.

High levels of electrophoretic variability observed for the M-line snails were most likely associated with interbreeding among stocks. Albino stocks of *B. glabrata* currently used may have separate origins and histories. The M-line was the result of laboratory selection to combine albinism and susceptibility. Another albino stock was isolated by Dr. Paul Thompson in 1953 from the field in Puerto Rico (J. Bruce, pers. comm.). This stock had been maintained by Drs. Henry van der Schalie (deceased) and Elmer Berry (retired) at the University of Michigan and John I. Bruce at the University of Massachusetts–Lowell. Both stocks of *B. glabrata* display susceptibility to *S. mansoni* and albinism; however, because these stocks had separate origins, there is no reason to expect a single underlying genetic control for these traits. The observation of high genetic heterogeneity and polymorphism and the occurrence of 3 alleles for some loci would be consistent with mixing and interbreeding these 2 stocks.

One of the advantages of defined laboratory stocks is their potentially more uniform response to experimental manipulation. Our information suggests that it would be useful to clarify the history of *Biomphalaria* stocks used in research so that stock designations can be uniformly applied to single genetic stocks of snails and so that the genetic integrity of particular stocks can be maintained.

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Table 4. Comparison of F_{st} for studies with multiple samples of some species of *Biomphalaria*.

Species	Number and location of samples	F_{st}	References
B. glabrata	5 M-line colonies	0.173	This study
B. glabrata	6 West Indian samples	0.805	Mulvey et al., 1988
B. glabrata	7 Puerto Rican samples		Mulvey and Vrijenhoek, 1982
B. straminea	4 Hong Kong samples	0.098	Woodruff et al., 1985
B. alexandrina	11 Egyptian samples	0.200	Graven, 1984
B. pfeifferi	12 Kenyan samples	0.589	Bandoni et al., 1990

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